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Concurrent degradation of tetrabromobisphenol A by *Ochrobactrum* sp. T under aerobic condition and estrogenic transition during these processes

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ABSTRACT

The effect of concurrent degradation of tetrabromobisphenol A (TBBPA) by the strain *Ochrobactrum* sp. T under aerobic condition was investigated. The results demonstrated that four extra energy source-addition systems still followed pseudo-first order kinetics. The addition of ethanol or glucose could promote the biodegradation ability of *Ochrobactrum* sp. T to TBBPA, and 90.1 percent and 77.5 percent of TBBPA (5 mg L^{-1}) could be removed with corresponding TBBPA half-lives of 26 and 36 h, respectively, after 96 h reaction. Comparatively, the degradation efficiency of the sole TBBPA system was only 72.9 percent under the same condition. In contrast, two other co-substrates 2,4,6-tribromophenol (TBP) and bisphenol A (BPA) showed a negative effect on the TBBPA biodegradation, and the degradation efficiencies of TBBPA were achieved as 44.7 percent and 67.4 percent, respectively. For the TBBPA+TBP system, the competitive inhibition for the TBBPA debromination was less than the inhibition of the toxicity to the bacterium. While for the TBBPA+BPA system, the degradation of TBBPA could be promoted at the beginning of the reaction, and was then inhibited slightly with further prolonging of reaction time. This is probably due to the substrates being oxidized, and BPA can consume partial oxygen and provide the electrons during the concurrent biodegradation process. In addition, although higher estrogenic activity could be detected for the debrominated intermediates in TBBPA co-degradation process than the original TBBPA, the estrogenicity of the whole system still decreased finally after 96 h degradation.

1. Introduction

Tetrabromobisphenol A (TBBPA) is one of the most widely used brominated flame retardants (BFRs) to effectively reduce the flammability of the final manufactured products around the world (de Wit, 2002). However, it can be emitted and pollute the environment via the use of all sorts of electrical products and the dismantlement of electrical wastes (Li et al., 2008; Ni et al., 2010). What is more, possible toxic effects of TBBPA such as endocrine disruption and acute toxicity to some aquatics have already been reported (Darnerud, 2003; Kuiper et al., 2007; Lilienthal et al., 2008; Kling and Forlin, 2009; Strain et al., 2009). Therefore, the degradation of TBBPA including phototransformation (Eriksson et al., 2004; Horikoshi et al., 2008) and biodegradation (Ronen and Abeliovich, 2000; Voordeckers et al., 2002; Gerecke et al., 2006) was carried out. Previous reports about the phototransformation were mainly focused on debromination, mineralization and identification of the degradation intermediates in aqueous solutions under UV-irradiation. And almost all of the biodegradations were limited by the different conditions between reductive debromination and oxidative mineralization. In our previous study, one bacterial strain *Ochrobactrum* sp. T with the ability to simultaneously debrominate and mineralize TBBPA was isolated (An et al., 2011), which can effectively solve the problem above. Dehalogenation, a process through which dehalogenation bacteria utilize halogenated compounds as election acceptors, can

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be enhanced or inhibited by presented other electron donors and acceptors (de Wit, 2002). So the study of the debromination intermediates which may serve as electron acceptors is also needed.

To the best of our knowledge, additional electron donor and acceptor are essential to the organics metabolism, especially the redox related process (Hong et al., 2007a, 2007b). Nevertheless, most studies on TBBPA biodegradation were mainly focused on the debromination kinetics (Arbeli and Ronen, 2003). Only one work reported the effect of the electron transfer system on the TBBPA anaerobic debromination (Arbeli et al., 2006), which suggested that the substrate TBBPA served as the electron acceptor, and ethanol was utilized as carbon and energy sources. This situation may not be proper for the aerobic degradation of TBBPA. According to our previous study (An et al., 2011), TBBPA aerobic degradation included two simultaneous processes of reduction and oxidation; oxygen is used to oxidize TBBPA first and the other oxidative intermediates consume the oxygen which is supposed to be a competitive electron acceptor of TBBPA, thus providing the reducing power and ensuring that reductive debromination of TBBPA occurs easily. So, to get a complete insight into the aerobic degradation process of TBBPA, the study of concurrent additional energy sources including TBBPA degradation intermediates in the degradation system is necessary.

As a potential toxic compound, the estrogenic activity of TBBPA is still under intensive debate. For instance, some previous studies reported that TBBPA probably possessed the estrogenic activity, showing inhibition of estrogen sulfotransferase in vitro (Hamers et al., 2006) or enhanced proliferation in an estrogen-dependent cell line (Kitamura et al., 2002). While some other studies indicated that the estrogen-like effect of TBBPA was insignificant (Samuelsen et al., 2001; Dorosh et al., 2011), its degradation intermediates might possess higher potential estrogenicity (Samuelsen et al., 2001; Uhnáková et al., 2011). Therefore, it is essential to evaluate the safety of the estrogenic transition during the TBBPA biodegradation process, and the estrogenic activity investigation of TBBPA concurrent biodegradation could provide more information for other brominated analogs of TBBPA.

To compare with the anaerobic debromination process, in this work, four additional energy sources were selected as the electron donors as well as carbon sources to investigate their effect on TBBPA biodegradation kinetics under aerobic condition. Furthermore, 2,4,6-tribromophenol (TBP) and bisphenol A (BPA) were employed as the concurrent substrate to determine the structure analog effect on TBBPA biodegradation, and also to confirm the mechanism of TBBPA aerobic degradation proposed in our previous work. The estrogenic transition was also investigated to measure the toxicity during the TBBPA concurrent biodegradation. As far as we know, this is the first study of TBBPA concurrent degradation carried out under aerobic condition.

2. Materials and methods

2.1. Chemicals and growth medium

TBBPA (purity: 97 percent) and TBP (99 percent) were purchased from Sigma-Aldrich. BPA (97 percent) was offered by Acros Organics (New Jersey, USA). All other chemicals (analytical grade reagents with more than 99 percent purity) used for the preparation of aqueous medium and biochemical experiments were obtained from Guangzhou Chemical Reagent Co., Inc., China. Our previous isolated bacterial strain *Ochrobactrum* sp. T (HM543185) (An et al., 2011) was employed for the concurrent degradation of TBBPA, and the growth medium for cell enrichment and mineral medium (MM) for degradation were all prepared according to Eriksson et al. (2004) and An et al. (2011).

2.2. Concurrent degradation of TBBPA

The strain *Ochrobactrum* sp. T was pre-cultured in the Luria-Bertani (LB) medium for 15 h, collected by centrifugation at 6000*g* for 3 min, and washed with the MM twice. The biodegradation experiments were performed by inoculating 25 mL of harvested cultures into 100 mL MM (in 250 mL shake flasks) containing 5 mg L^{-1} TBBPA at 35 °C, pH 7.0, and 200 rpm for 96 h. The following compounds (g L^{-1}) were used as the carbon sources and electron donors: ethanol 1.0, acetate 1.66, pyruvate 1.0 and glucose 0.5. The pH values of acetate and the pyruvate addition system were adjusted to about 7.0 by 1 mol L^{-1} HCl and 1 mol L^{-1} NaOH, respectively. The growth curve of *Ochrobactrum* sp. T in each energy source was determined by the optical density at 600 nm (OD_{600}) which was measured using a spectrophotometer after 2–3 s vigorous vortexing. For concurrent substrate degradation of TBBPA, TBP and BPA were separately tested at 5.0 mg L^{-1} (Arbeli et al., 2006). All experiments were conducted in triplicate.

2.3. Estrogenic activity assay

The transition of estrogenic activity was detected during the TBBPA biodegradation process as well as during the concurrent degradation process (TBBPA+TBP and TBBPA+BPA, respectively) by measuring the β-galactosidase activity of a recombination yeast cell. The experiments steps and calculation method were all similar to those of our previous study (Li et al., 2012). 17β-estradiol (E2) was still used as a standard control, and ten E2 concentrations 10^{-11}, 2.5×10^{-11}, 5×10^{-11}, 10^{-10}, 2.5×10^{-10}, 5×10^{-10}, 10^{-9}, 10^{-8}, 5×10^{-8}, and 10^{-7} mol L^{-1} were chosen to establish the dose–response curve.

2.4. Analytical methods

Concentrations of TBBPA, BPA and TBP were all measured by high performance liquid chromatography (HPLC) (Agilent 1200 HPLC) equipped with a DAD detector. The detailed detection conditions were similar to those of our previous work (An et al., 2011; Zu et al., 2012). Wavelengths of 230, 280 and 286 nm were used to detect TBBPA, BPA and TBP, respectively. The eluent was a mixed solution of 80 percent methanol, eighteen percent ultra-pure water, and two percent glacial acetic acid at a flow rate of 1 mL min^{-1}. The retention time of TBBPA was 9.644 min, while TBP and BPA showed retention time of 8.394 and 3.530 min, respectively.

3. Results

3.1. Effect of energy sources on TBBPA biodegradation

It is well known that most of the redox power needed for the xenobiotics metabolized by microorganisms is offered through the electron transfer chain. Therefore, four energy sources such as ethanol, acetate, pyruvate and glucose were employed as the additional electron donors as well as carbon sources during the *Ochrobactrum* sp. T degradation of TBBPA process (Fig. 1). Results showed that after 96 h reaction, the highest TBBPA degradation efficiency of 90.1 percent was achieved in the ethanol-addition system, and the value of 77.5 percent in the glucose-addition system. Both of them are higher than the control sample (TBBPA only) value of 72.9 percent. In contrast, lower TBBPA biodegradation efficiencies of 57.9 percent and 48.7 percent were obtained in acetate- and pyruvate-addition systems, respectively. It can also be found that the biodegradation efficiency of TBBPA increased with degradation time in each energy source-addition system within

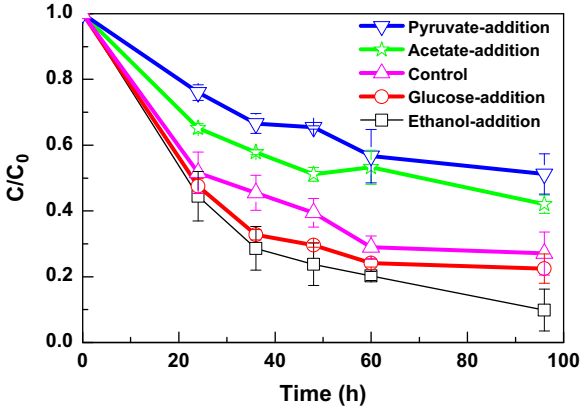

Fig. 1. Biodegradation efficiency of TBBPA associated with different additional energy sources.

96 h, although the increase was not so obvious in the glucose-addition and control samples after 60 h, indicating that no noticeable promotion effect existed with the prolongation of the reaction time after 60 h in these two systems. The strain's growth curves on different energy sources were also investigated (Fig. S1). It can be seen that *Ochrobactrum* sp. T was enriched best in the pyruvate-addition system and the highest OD_{600} value was 0.416 at 24 h, while the poorest growth was achieved in acetate-addition system followed by the control system without any additional energy sources. This may indicate that *Ochrobactrum* sp. T used pyruvate as the carbon source rather than electron donor during the TBBPA biodegradation.

To further investigate the effect of these energy sources, the negative index of least-square regression by the return was employed to fit each biodegradation process. The results showed that the decrease of TBBPA concentration in all five degradation systems matched with the negative index exponential curves very well (Fig. 2), indicating that these energy source-addition degradations still obeyed pseudo-first-order-kinetics. The kinetic equations by linear fitting with $\ln C_0/C_t = Kt$ are listed in Table 1. Here C_0 and C_t are the initial concentration of TBBPA fixed at 5 mg L^{-1} and the detected concentration at t, respectively; K and t are the biodegradation rate constant (h^{-1}) and the degradation time (h), respectively. The results showed that the half-lives of TBBPA in the ethanol-addition, glucose-addition, control, acetate-addition and pyruvate-addition system increased from 26 to 88 h, revealing that the biodegradation of TBBPA could be inhibited by the addition of acetated and pyruvate.

3.2. Effect of concurrent substrates on TBBPA biodegradation

In our previous work, TBP and BPA were detected as the metabolites during *Ochrobactrum* sp. T degradation of the TBBPA process (An et al., 2011). To further investigate the effect of these intermediates on TBBPA biodegradation as well as to validate the previously proposed TBBPA biodegradation mechanism by *Ochrobactrum* sp. T, these two compounds were further chosen as the concurrent substrate (An et al., 2011). It showed that the addition of TBP obviously inhibited the *Ochrobactrum* sp. T from degrading TBBPA, and only 44.7 percent TBBPA was removed after 96 h reaction, while 72.9 percent of TBBPA was degraded in the TBBPA alone system (control sample) (Fig. 3). It should also be noted that the degradation efficiencies of TBBPA in the TBBPA+TBP system slowly increased from 41.9 to 43.7 percent with the increase of the reaction time from 24 to 60 h, indicating that the TBBPA degradation predominantly occurred in the first 24 h, and no remarkable promotion of TBBPA degradation could be achieved by further extending the reaction time to 96 h. It could also be noted that the strain *Ochrobactrum* sp. T possessed limited ability to degrade TBP in the TBBPA+TBP system, and only 4.7 percent of TBP was removed within 24 h. With the prolonging of reaction time further to 96 h, the degradation efficiencies were not increased; on the contrary, the concentration of TBP increased slightly, and the values of C/C_0 were around 100 percent. The increased TBP concentration may originate from TBBPA degradation which can be confirmed by Eriksson (1998) as well as ours (An et al., 2011).

The degradation kinetics of TBBPA associated with another concurrent substrate BPA is demonstrated in Fig. 4. After 96 h reaction, 67.4 percent of TBBPA was removed in the TBBPA+BPA system, which was about 6 percent lower than that of the TBBPA alone system (control sample). However, the degradation efficiencies of TBBPA as well as BPA in the co-metabolism system fluctuate before this stage. For the TBBPA, the curve can be described in four stages: within 24 h, the concentration of TBBPA showed a rapid decrease with the degradation efficiency of 52.2 percent while that of the control sample was just 48.3 percent. At the stage of 24–48 h, a temporary stagnation emerged, and the degradation efficiency was only up to 52.9 percent at 48 h while that of the control sample was 60.5 percent. In addition, an obvious crossing

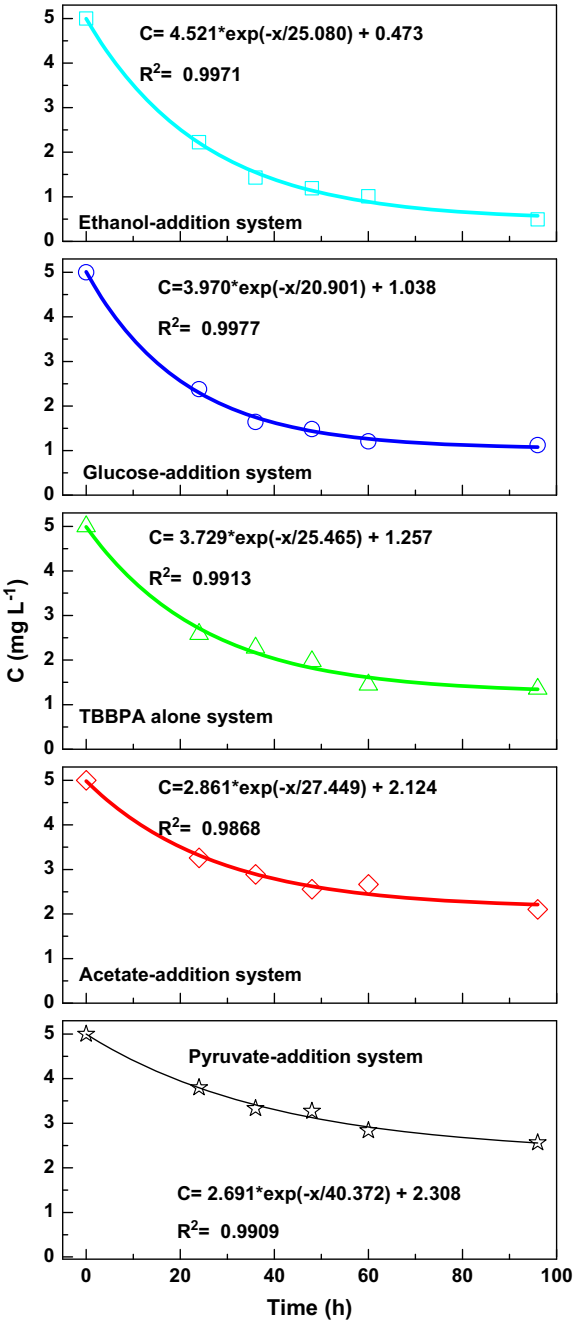

Fig. 2. Negative index of least-square regression results of TBBPA biodegradation with different additional energy sources.

Table 1
Degradation kinetic equations of TBBPA with different additional energy sources.

Additional energy source	Kinetic equation	R^2	K (h^{-1})	Half-life (h)
Ethanol	$\ln C_t = -0.02656t + 1.609$	0.9805	0.02656	26
Glucose	$\ln C_t = -0.0194t + 1.609$	0.9253	0.0194	36
Control	$\ln C_t = -0.01633t + 1.609$	0.9522	0.01633	42
Acetate	$\ln C_t = -0.01026t + 1.609$	0.9291	0.01026	68
Pyruvate	$\ln C_t = -0.00789t + 1.609$	0.9675	0.00789	88

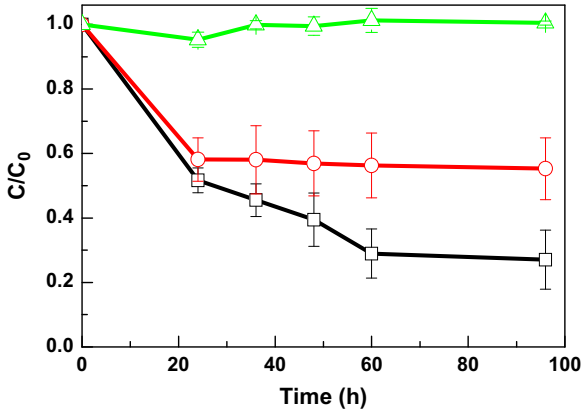

Fig. 3. Effect of TBP on TBBPA concurrent biodegradation: (△) TBP degradation efficiency in the TBP+TBBPA system; (○) TBBPA degradation efficiency in the TBP+TBBPA system; (□) TBBPA degradation efficiency in the TBBPA alone system.

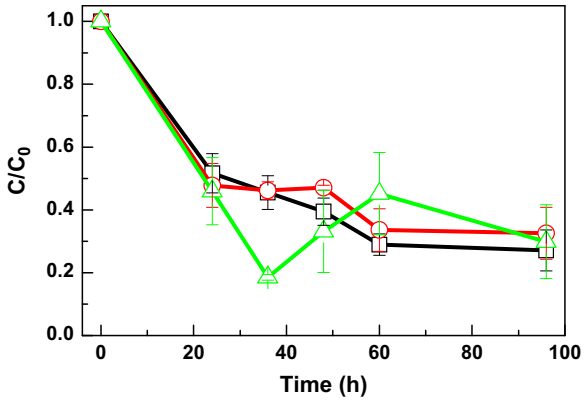

Fig. 4. Effect of BPA on TBBPA concurrent biodegradation: (△) BPA degradation efficiency in the BPA+TBBPA system; (○) TBBPA degradation efficiency in the BPA+TBBPA system; (□) TBBPA degradation efficiency in the TBBPA alone system.

can be discerned at 36 h between the concurrent degradation and the control one. During the third stage (48–60 h), the degradation efficiency swiftly increased to 66.4 percent at 60 h, with no remarkable decrease of TBBPA from 60 to 96 h (the fourth stage). Comparatively, Unlike the TPB, the evolution curve of BPA in the TBBPA+BPA system can be effectively degraded by this TBBPA-degrading strain *Ochrobactrum* sp. T. This result doubly confirmed our previously proposed one-step process for debromination and aerobic mineralization of TBBPA mechanisms by this strain (An et al., 2011). As seen, the degradation efficiency of BPA could reach 81.5 percent at 36 h, even higher than that of the TBBPA degradation in the same system. However, as an intermediate of TBBPA degradation, BPA was then accumulated from 36 to 60 h, and the degradation efficiency reduced to 54.8 percent at 60 h. After the accumulation stage, BPA concentration decreased again and 70.1 percent of BPA could be degraded at 96 h.

3.3. Estrogenic transition during concurrent degradation of TBBPA

Furthermore, to better understand the process of the TBBPA concurrent degradation, the estrogenic activity transition was also evaluated using an estrogenic activity assay kit. According to the steps of the kit, the calibration curve of 17β-estradiol was investigated first (Fig. S2). The result showed that at the concentration of 0.01–50 nmol L^{-1}, the dose–response curve of 17β-estradiol fitted the sigmoidal response very well (R^2=0.9956), which validated the

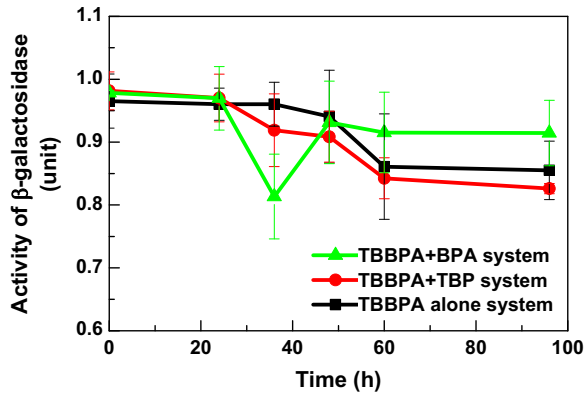

Fig. 5. The estrogenic transition during the concurrent degradation of TBBPA.

effectiveness of the method. The result of the estrogenic transition on the concurrent biodegradation of TBBPA is demonstrated in Fig. 5. It shows that within 96 h biodegradation, the estrogenicities decreased in all three TBBPA degradation systems. In the TBBPA alone biodegradation process, the estrogenicity decreased very slowly from 0.97 to 0.94 U during the first 48 h. An obvious decreasing occurred within 48–60 h, and the estrogenicity reduced to 0.86 U at 60 h. With further degradation time, the estrogenicity leveled off. This transition tendency is very similar to that of the TBBPA co-degradation with TBP. That is, in this TBBPA+TBP system, the estrogenicity almost did not decrease during first 24 h, and gradually decreased from 0.97 to 0.84 U with the increase of reaction time from 24 to 60 h, and then the lowest value of 0.83 U was achieved at 96 h degradation. Comparatively, the estrogenicity transition of TBBPA co-degradation with BPA showed a notable difference. The enzyme activity decreased quickly from 0.97 to 0.81 U within 24–36 h, and then increased to 0.93 U at 48 h. A little reducing occurred beyond 48 h.

Comparing Figs. 5 and S1 , it seems that these TBBPA systems can induce the activity of β-galactosidase much stronger than E2. However, it is worth mentioning that the estrogenic activity of a compound is not only determined by the compound itself, but also by its concentration, which already has been proved in many peer-reviewed published papers. For instance, E2 exhibits very potent estrogenic activity even at a very low concentration (Purdom et al., 1994; Gaido et al., 1997; Ohko et al., 2002; Wright-Walters and Volz, 2009; Vandenberg et al., 2012). In our case, the dose–response curve of E2 (Fig. S2) was investigated as the standard control to verify the effectivity of the method and whether it fitted the sigmoidal response, and ten concentrations of E2 10^{-11}, 2.5×10^{-11}, 5×10^{-11}, 10^{-10}, 2.5×10^{-10}, 5×10^{-10}, 10^{-9}, 10^{-8}, 5×10^{-8}, and 10^{-7} mol L^{-1} were chosen to establish the dose–response curve. The concentration of TBBPA is 5 mg L^{-1} (9.19×10^{-6} mol L^{-1}), which is about two orders of magnitude higher than the highest concentration of E2. Therefore, this result only shows that TBBPA at this concentration possesses estrogenic activity and it may not be very proper to give a conclusion that TBBPA systems have stronger estrogenic activity than that of E2.

4. Discussion

As for the energy source, ethanol showed the best active effect in straining *Ochrobactrum* sp. T on the aerobic biodegradation of TBBPA (Fig. 1). And this result is similar to a previous study which reported that the debromination bacteria had the fastest debromination activity when ethanol was added in the system (Arbeli

et al., 2006). This is because ethanol usually could be employed as the electron donor as well as the carbon source in the organics metabolism (Hallin and Pell, 1998; Adrian et al., 2003; Davis et al., 2004). But in this study, since both TBBPA debromination and mineralization occurred under the aerobic condition, the ethanol serving as an electron donor should be more prominent to the reductive debromination step than under the anaerobic condition, and thus promote the biodegradation. This could also be confirmed by the strain's growth curve on different energy sources (Fig. S1). The OD_{600} values under pyruvate and glucose were all higher than that of ethanol, but the degradation efficiency was still lower than that of ethanol, indicating the main effect of an electron donor. For glucose, the effect of carbon source may be involved, but the contribution of electron donor was not so much as that of ethanol. The highest OD_{600} value was achieved in the pyruvate-addition system, which suggested that pyruvate was a favorable carbon source for *Ochrobactrum* sp. T, but this would result in a lower utilization for TBBPA because they were all optional carbon sources to the strain. The inhibition effect by acetate and pyruvate was a little different from that in Arbeli et al. (2006), which reported that acetate and pyruvate could enhance the debromination of TBBPA. This may be due to the different degradation conditions employed. For instance, our degradation was carried out under aerobic condition, while they performed the reaction under anaerobic condition. In addition, some studies also showed that as an electron donor, acetate could effectively promote the degradation of organics in a H_2 combination system (He et al., 2002), while acetate alone may not cause an obvious promotion of the process (Arbeli et al., 2006).

From the results of concurrent substrate effect on the biodegradation of TBBPA (Figs. 3 and 4), it can be found that either TBP or BPA could inhibit the reaction, although the ways are different. For both bromine atom containing chemicals, TBP could be a competitive inhibitor to the debromination process of TBBPA, which can be proved by the result that the degradation efficiency of TBBPA in the TBBPA+TBP system is lower than that in the TBBPA alone system within 24 h degradation (Fig. 3). However, after 24 h, almost no degradation of TBBPA was detected in the TBBPA+TBP system, suggesting that the toxicity of TBP may play a leading role during the TBBPA biodegradation process. Since TBP is toxic to some TBBPA debromination microorganisms (Arbeli et al., 2006) it can be proposed that TBP probably acted more like a cell inhibitor than like a competitive inhibitor to TBBPA degradation in this study.

As a TBBPA debrominated by product, BPA showed a slight negative effect to TBBPA degradation in the TBBPA+BPA system after 36 h, but at the beginning of 24 h, the two degradation curves of TBBPA alone and BPA-addition system almost coincided, and the degradation of latter is even slightly faster than that of former. Furthermore, this result may offer another proof to the proposed pathways of TBBPA biodegradation by *Ochrobactrum* sp. T in our previous paper: the crucial process to ensure the possibility of simultaneous debromination and mineralization of TBBPA was the formation of oxidative intermediates to consume the oxygen first, and then the reductive debromination could occur subsequently (An et al., 2011). Being similar to the process described above, the concurrent substrate BPA could be simultaneously degraded by strain *Ochrobactrum* sp. T (Fig. 4), and this course may consume the oxygen and also provide the electrons for reductive debromination of TBBPA. Therefore, the biodegradation could be enhanced to some extent which could explain the rapid reaction before 24 h. With the further increase of the degradation time, more BPA was accumulated and high BPA concentration may inactivate the strain, and subsequently will decrease the degradation efficiency of TBBPA as reported by some BPA biodegradation studies (Yamanaka et al., 2007; Zhang et al., 2007).

In addition, the investigation of estrogenic transition on the concurrent degradation of TBBPA provided an insight into the estrogen-like effect of TBBPA and its biodegradation intermediates. Results showed that, at the beginning of the reaction, TBBPA was degraded swiftly in all three systems (TBBPA alone, TBBPA+TBP and TBBPA+BPA system; Figs. 3 and 4), while the estrogenicities were almost not decreasing accordingly (Fig. 5). The result indicated that the produced TBBPA intermediates at this stage still possessed the equivalent degree of estrogen-like effect as that of TBBPA. This conclusion is accordant with a previous study showing that the estrogenic effect of TBBPA degradation products is even more significant than TBBPA itself (Uhnáková et al., 2011). Moreover, it should be noted that in the TBBPA+BPA system, estrogenic transition (Fig. 5) exhibited a trend similar to the change of BPA degradation efficiency (Fig. 4). It revealed that the estrogen-like effect of BPA was the major contributor to the estrogenicity of the whole reaction system, indicating the highest estrogenic activity of BPA in all the TBBPA debromination products. These conclusions suggest that for the debrominated products of TBBPA, the estrogen-like potential decreases with increasing number of bromo-substitutions which is consistent with a previous study (Samuelsen et al., 2001).

5. Conclusions

The present work studied the concurrent degradation of TBBPA with four extra energy sources and two degradation intermediates of TBBPA by the strain *Ochrobactrum* sp. T under aerobic condition. The results well illustrated that ethanol-, glucose-, acetate- and pyruvate-addition systems followed pseudo-first-order-kinetics. Ethanol and glucose addition promoted the biodegradation of TBBPA, while the addition of acetate and pyruvate inhibited the biodegradation. As for the effects of the two debromination intermediates, TBP had negative effect to the degradation of TBBPA, while BPA had a little stimulation before 36 h, and then had a slight negative effect to TBBPA degradation beyond 36 h. So it is conservatively proposed that TBP could be a competitive inhibitor or a cell inhibitor to TBBPA debromination at different biodegradation stages, and for the first time verifies the possibility of TBBPA concurrent biodegradation in the aerobic condition under the influence of BPA. The study about the estrogenic transition in TBBPA co-degradation process proved that BPA was a compound with the highest estrogenic activity during the TBBPA degradation process by *Ochrobactrum* sp. T, but the estrogenic activity can be decreased in this process despite these high estrogenicity intermediates. Knowing the mechanism and the estrogen property of the concurrent biodegradation process under aerobic condition is essential for the biodegradation of TBBPA. Furthermore, elucidation in the concurrent biodegradation process of the two aspects should have important assistance for remediation of TBBPA.

Acknowledgments

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ecoenv.2014.03.015.

References

Adrian, N.R., Arnett, C.M., Hickey, R.F., 2003. Stimulating the anaerobic biodegradation of explosives by the addition of hydrogen or electron donors that produce hydrogen. Water Res. 37, 3499–3507.

An, T.C., Zu, L., Li, G.Y., Wan, S.G., Mai, B.X., Wong, P.K., 2011. One-step process for debromination and aerobic mineralization of tetrabromobisphenol-A by a novel *Ochrobactrum* sp. T isolated from an e-waste recycling site. Bioresour. Technol. 102, 9148–9154.

Arbeli, Z., Ronen, Z., 2003. Enrichment of a microbial culture capable of reductive debromination of the flame retardant tetrabromobisphenol-A, and identification of the intermediate metabolites produced in the process. Biodegradation 14, 385–395.

Arbeli, Z., Ronen, Z., Diaz-Baez, M.a.C., 2006. Reductive dehalogenation of tetrabromobisphenol-A by sediment from a contaminated ephemeral streambed and an enrichment culture. Chemosphere 64, 1472–1478.

Darnerud, P.O., 2003. Toxic effects of brominated flame retardants in man and in wildlife. Environ. Int. 29, 841–853.

Davis, J.L., Wani, A.H., O'Neal, B.R., Hansen, L.D., 2004. RDX biodegradation column study: comparison of electron donors for biologically induced reductive transformation in groundwater. J. Hazard. Mater. 112, 45–54.

de Wit, C.A., 2002. An overview of brominated flame retardants in the environment. Chemosphere 46, 583–624.

Dorosh, A., Děd, L., Elzeinová, F., Pěknicová, J., 2011. Assessing oestrogenic effects of brominated flame retardants hexabromocyclododecane and tetrabromobisphenol A on MCF-7 cells. Folia Biol. 56, 35–39.

Eriksson, J., 1998. Decomposition of tetrabromobisphenol A in the presence of UV-light and hydroxyl radicals. Organohalogen Compd. 35, 419–422.

Eriksson, J., Rahm, S., Green, N., Bergman, A., Jakobsson, E., 2004. Photochemical transformations of tetrabromobisphenol A and related phenols in water. Chemosphere 54, 117–126.

Gaido, K.W., Leonard, L.S., Lovell, S., Gould, J.C., Babai, D., Portier, C.J., McDonnell, D.P., 1997. Evaluation of chemicals with endocrine modulating activity in a yeast-based steroid hormone receptor gene transcription assay. Toxicol. Appl. Pharmacol. 143, 205–212.

Gerecke, A.C., Giger, W., Hartmann, P.C., Heeb, N.V., Kohler, H.-P.E., Schmid, P., Zennegg, M., Kohler, M., 2006. Anaerobic degradation of brominated flame retardants in sewage sludge. Chemosphere 64, 311–317.

Hallin, S., Pell, M., 1998. Metabolic properties of denitrifying bacteria adapting to methanol and ethanol in activated sludge. Water Res. 32, 13–18.

Hamers, T., Kamstra, J.H., Sonneveld, E., Murk, A.J., Kester, M.H.A., Andersson, P.L., Legler, J., Brouwer, A., 2006. In vitro profiling of the endocrine-disrupting potency of brominated flame retardants. Toxicol. Sci. 92, 157–173.

He, J.Z., Sung, Y., Dollhopf, M.E., Fathepure, B.Z., Tiedje, J.M., Loffler, F.E., 2002. Acetate versus hydrogen as direct electron donors to stimulate the microbial reductive dechlorination process at chloroethene-contaminated sites. Environ. Sci. Technol. 36, 3945–3952.

Hong, Y., Guo, J., Xu, Z., Mo, C., Xu, M., Sun, G., 2007a. Reduction and partial degradation mechanisms of naphthylaminesulfonic azo dye amaranth by *Shewanella decolorationis* S12. Appl. Microbiol. Biotechnol. 75, 647–654.

Hong, Y., Xu, M., Guo, J., Xu, Z., Chen, X., Sun, G., 2007b. Respiration and growth of Shewanella decolorationis S12 with an Azo compound as the sole electron acceptor. Appl. Environ. Microbiol. 73, 64–72.

Horikoshi, S., Miura, T., Kajitani, M., Horikoshi, N., Serpone, N., 2008. Photodegradation of tetrahalobisphenol-A (X=Cl, Br) flame retardants and delineation of factors affecting the process. Appl. Catal. B: Environ. 84, 797–802.

Kitamura, S., Jinno, N., Ohta, S., Kuroki, H., Fujimoto, N., 2002. Thyroid hormonal activity of the flame retardants tetrabromobisphenol A and tetrachlorobisphenol A. Biochem. Biophys. Res. Commun. 293, 554–559.

Kling, P., Forlin, L., 2009. Proteomic studies in zebrafish liver cells exposed to the brominated flame retardants HBCD and TBBPA. Ecotoxicol. Environ. Saf. 72, 1985–1993.

Kuiper, R.V., Canton, R.F., Leonards, P.E.G., Jenssen, B.M., Dubbeldam, M., Wester, P.W., van den Berg, M., Vos, J.G., Vethaak, A.D., 2007. Long-term exposure of European flounder (*Platichthys flesus*) to the flame-retardants tetrabromobisphenol A (TBBPA) and hexabromocyclododecane (HBCD). Ecotoxicol. Environ. Saf. 67, 349–360.

Li, G.Y., Zu, L., Wong, P.K., Hui, X.P., Lu, Y., Xiong, J.K., An, T.C., 2012. Biodegradation and detoxification of bisphenol A with one newly-isolated strain *Bacillus* sp. GZB: kinetics, mechanism and estrogenic transition. Bioresour. Technol. 114, 224–230.

Li, J., Ma, M., Wang, Z.J., 2008. A two-hybrid yeast assay to quantify the effects of xenobiotics on thyroid hormone-mediated gene expression. Environ. Toxicol. Chem. 27, 159–167.

Lilienthal, H., Verwer, C.M., van der Ven, L.T.M., Piersma, A.H., Vos, J.G., 2008. Exposure to tetrabromobisphenol A (TBBPA) in Wistar rats: neurobehavioral effects in offspring from a one-generation reproduction study. Toxicology 246, 45–54.

Ni, H.-G., Zeng, H., Tao, S., Zeng, E.Y., 2010. Environmental and human exposure to persistent halogenated compounds derived from e-waste in China. Environ. Toxicol. Chem. 29, 1237–1247.

Ohko, Y., Iuchi, K.I., Niwa, C., Tatsuma, T., Nakashima, T., Iguchi, T., Kubota, Y., Fujishima, A., 2002. 17 beta-estrodial degradation by TiO_2 photocatalysis as means of reducing estrogenic activity. Environ. Sci. Technol. 36, 4175–4181.

Purdom, C.E., Hardiman, P.A., Bye, V.J., Eno, N., Tyler, C., Sumpter, J., 1994. Estrogenic effects of effluents from sewage treatment works. Chem. Ecol. 8, 275–285.

Ronen, Z., Abeliovich, A., 2000. Anaerobic–aerobic process for microbial degradation of tetrabromobisphenol A. Appl. Environ. Microbiol. 66, 2372–2377.

Samuelsen, M., Olsen, C., Holme, J.A., Meussen-Elholm, E., Bergmann, A., Hongslo, J.K., 2001. Estrogen-like properties of brominated analogs of bisphenol A in the MCF-7 human breast cancer cell line. Cell Biol. Toxicol. 17, 139–151.

Strain, G.M., Banasik, M., Hardy, M., Stedeford, T., 2009. Tetrabromobisphenol A (TBBPA) and model-derived risks for neurobehavioral effects in offspring from a one-generation reproduction study. Toxicology 260, 155–157.

Uhnáková, B., Ludwig, R., Pěknicová, J., Homolka, L., Lisá, L., Šulc, M., Petříčková, A., Elzeinová, F., Pelantová, H., Monti, D., Křen, V., Haltrich, D., Martínková, L., 2011. Biodegradation of tetrabromobisphenol A by oxidases in basidiomycetous fungi and estrogenic activity of the biotransformation products. Bioresour.Technol. 102, 9409–9415.

Vandenberg, L.N., Colborn, T., Hayes, T.B., Heindel, J.J., Jacobs, D.R., Lee, D.H., Shioda, T., Soto, A.M., vom Saal, F.S., Welshons, W.V., Zoeller, R.T., Myers, J.P., 2012. Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. Endocr. Rev. 33, 378–455.

Voordeckers, J.W., Fennell, D.E., Jones, K., Haggblom, M.M., 2002. Anaerobic biotransformation of tetrabromobisphenol A, tetrachlorobisphenol A, and bisphenol A in estuarine sediments. Environ. Sci. Technol. 36, 696–701.

Wright-Walters, M., Volz, C., 2009. Municipal wastewater concentrations of pharmaceutical and xeno-estrogens: wildlife and human health implications. In: Proceedings of the National Conference on Environmental Science and Technology, pp. 103–113.

Yamanaka, H., Moriyoshi, K., Ohmoto, T., Ohe, T., Sakai, K., 2007. Degradation of bisphenol A by Bacillus pumilus isolated from kimchi, a traditionally fermented food. Appl. Biochem. Biotechnol. 136, 39–51.

Zhang, C., Zeng, G.M., Yuan, L., Yu, J., Li, J.B., Huang, G.H., Xi, B.D., Liu, H.L., 2007. Aerobic degradation of bisphenol A by *Achromobacter xylosoxidans* strain B-16 isolated from compost leachate of municipal solid waste. Chemosphere 68, 181–190.

Zu, L., Li, G.Y., An, T.C., Wong, P.K., 2012. Biodegradation kinetics and mechanism of 2,4,6-tribromophenol by *Bacillus* sp. GZT: a phenomenon of xenobiotic methylation during debromination. Bioresour. Technol. 110, 153–159.